



Protocol for genotyping LE-Rosa26Tm1(LSL-Cas9D10A)Ottc transgenic rats January 17, 2019

Genomic DNA Preparation by Macherey-Nagel Tissue Spin Columns

Using this kit according to the manufacturer's protocol is the preferred way for preparing genomic DNA at OTTC when it is intended for ddPCR (i.e. copy-number quantification). Typically, 10 to 90 ng of genomic DNA are used in a 25uL PCR reaction.

General PCR reaction setup:

- 12.0 uL 2x Q5 master mix (New England Biolabs)
- 12.0 uL 2x specific oligos (1 uM Forward + 1uM Reverse; in water)
- 1.0 uL genomic DNA
- 25.0 uL PCR reaction

PCR Program CR1943		
<u>Line</u>	<u>Temp</u>	<u>Time</u>
Step 1	98oC	HOLD (hot start)
Step 2	98oC	30 sec
Step 3	98oC	10 sec
Step 4	60oC	30 sec
Step 5	72oC	2 min
Step 6	Go to Step 2	Repeat 34x
Step 7	72oC	5 min
Step 8	12oC	HOLD

Primer Sequences

<u>Primer Name</u>	<u>Primer Sequence (5' ... 3')</u>	<u>Amplicon</u>
NeoR F764	AGCGCATCGCCTTCTATCGC	3' junction
Rosa26 R67001	TTCTGCATTCCAGAAGGAAGTAAGTTTATAGAG	3' junction

3' junction

These oligos (NeoR F764 and Rosa26 R67001) produce a 1515 bp amplicon and work with Q5 polymerase and program CR1943. This assay is not suitable for genotyping the LSL-Cas9 rat that was also developed by NIDA/OTTC.

This protocol was updated on 01-17-2019 by CR.

Any questions regarding protocol, contact nidatransgenicprojects@mail.nih.gov.